

EXHIBIT 25

6 The Human Health programme in the Faroe Islands 1985-2001



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6.1 INTRODUCTION

Research on prenatal exposure to seafood contaminants and the potential effects on the foetus has been performed in the Faroes since 1985. This research has been carried out as a cooperation between the Department of Occupational and Public Health in the Faroese Hospital System and the Department of Environmental Medicine of the University of Southern Denmark. In addition to funding from the Danish Environmental Protection Agency and the Danish Medical Research Council, substantial support has been obtained from the U.S. National Institutes of Health and the European Commission.

In this report we will present preliminary results of studies financed by Arctic Monitoring and Assessment Program, under the following headings:

- "Effects of marine pollutants on the development of Faroese children" including 656 deliveries from December 1997 to February 2000. A description of the cohort characteristics, exposure to contaminants, obstetric optimality, neurological optimality and associations between exposures and potential effects will be presented. (6.2)
- "Dietary Survey of Faroese Women in the Third Trimester of Pregnancy" including 150 women October 2000 to October 2001. A description of contaminant levels in blood and main characteristics of the Faroese diet will be presented. (6.3)
- "Exposure to Seafood Contaminants and Immune Response in Faroese Children". The study is not yet finished, but the results of this study will be available in the near future. (6.4)
- "Exposure to POPs, 1985-2001" In this concluding section we will present exposure data from the above mentioned project as well as from other projects previously reported elsewhere. (6.5)

6.2 EFFECTS OF MARINE POLLUTANTS ON DEVELOPMENT IN FAROESE CHILDREN

6.2.1 Introduction

Consumption of marine food in preference to other food is generally considered to be healthy. But different contaminants such as persistent organic pollutants (POP) and heavy metals – in this case methyl mercury – might outweigh the advantages. The Faroese are known to be heavily dependent on seafood. Thus the exposure to some pollutants is very high in this population. Of special concern for the Faroese population are the following substances:

Organochlorine compounds, such as polychlorinated biphenyls (PCB) and chlorinated pesticides (4,4'-DDT, and its most stable daughter compound 4,4'-DDE, trans-nonachlor, β -HCH, HCB), have long been problematic in the environment for a number of reasons. Many of them (like DDT) have endocrine activity, therefore called hormonally active agents (HAAs). The term organochlorine refers to chemical compounds that have a chlorinated hydrocarbon structure; that is, they are formed from atoms of hydrogen, carbon, and chlorine. Although their effect may be much weaker than the body's natural hormones (like oestrogens, androgens, and thyroid hormones), they are nonetheless suspected of disrupting the endocrine system, resulting in harmful effects like reproductive and developmental defects. In humans, an association between breast cancer risk and PCB and DDT concentrations in mammary adipose tissues or plasma lipids could be shown in epidemiological studies (Dewailly et al., 1994). They are suspected to promote the progression of certain hormonally responsive tumors of the breast and uterus, especially DDT, β -HCH, HCB. According to animal research, organochlorines may be able to modulate immune response. Most of the substances have been banned for years, but due to their longevity in the environment, PCB, dieldrin, DDT, chlordane, aldrin, kepone, mirex, endrin, and toxaphene still constitute a public health problem. Human exposure to these contaminants is mainly through the consumption of food, particularly food caught in contaminated waters.

Polychlorinated biphenyls (PCBs) are divided into two main classes of congeners, the ortho-substituted and the non-ortho-substituted, which differ in their toxicology. The number of chloride atoms in the ortho-position of the two phenyl rings of the PCBs is responsible for the strength of the dioxin-like effect of the respective congener: the fewer the atoms in the ortho-position, the higher the dioxin-like toxicity. Thus, the planar or non-ortho congeners (IUPAC-numbers 77, 126, and 169) have a higher dioxin-like toxicity than the mono-ortho congeners (IUPAC-numbers 105, 114, 118, 123, 156, 157, 167). The toxic effect of PCBs and dioxins is mediated through binding to the cytosolic arylhydrocarbon (Ah)-receptor (Poland et al., 1992). The toxicity of the di-ortho PCBs (IUPAC-numbers 138, 153, 170, 180) depends on different mechanisms that are still being explored.

4,4'-dichlorodiphenyltrichloroethane (DDT) was used as an insecticide in agriculture. Nowadays it is banned in Europe, but it is still important in countries with serious mosquito borne disease problems such as malaria.

4,4'-dichlorodiphenyltrichloroethene (DDE) is the most stable daughter compound of DDT. DDE itself is persistent and bio-accumulating. Both

substances accumulate in fatty tissue. DDT can mimic the effects of oestrogen, and DDE can be anti-androgenic (AMAP, 1997).

Trans-nonachlor is a breakdown product of a mixture of different compounds, called chlordane, which is used as pesticide. It has major effects on the liver, the nervous system, and the immune system. It is also a probable human carcinogen.

Hexachlorobenzene (HCB) is a by-product in the production of a large number of chlorinated compounds. It was also used as a fungicide.

Hexachlorocyclohexanes are a group of compounds that are primarily used as pesticides. The most toxic form, gamma-hexachlorocyclohexane, is also called lindane. Due to its persistence, beta-hexachlorocyclohexane (β -HCH) usually is measured (AMAP, 1997). Effects of HCB and β -HCH are similar to those of the other mentioned pesticides (see the paragraph about trans-nonachlor), they all are excreted via breast milk.

An overview of the range of effects of organochlorines is given here:

- adverse neurobehavioral effects, neurotoxic effects (Seegal et al., 1992).
- Ah-receptor mediated effects are growth reduction (body weight reduction)
- immunologic effects are suspected (Tryphonas, 1998), but available data are inadequate to support any definitive conclusion (Committee on Hormonally Active Agents in the Environment, 2001)
- because of their structural similarity to steroid hormones they may interfere with endocrine function, such as thyroid function (Porterfield, 2000) and reproductive system (some PCB congeners showed a weak estrogen-like effect (Toppari et al., 1996); several hydroxy-PCB congeners exhibit oestrogen-receptor antagonistic effects (Committee on Hormonally Active Agents in the Environment, 2001); 4,4'-DDE acts as a weak antiandrogenic agent (AMAP, 1997).

Musk xylene, a synthetic aromatic substance, is often used in fragrances and soaps as a substitute for natural musk. It belongs to the group of nitromusk compounds. The main environmental intake of musk xylene occurs via consumption of fish and drinking water as well as the use of body care and perfumed household products. Musk xylene is stable against biological and chemical degradation and is highly lipophilic (Kafferlein et al., 1998). A genotoxic risk for humans has been assumed (Mersch-Sundermann et al., 1996).

Methylmercury (MeHg) is a well-established neurotoxicant that can cause serious adverse effects on the development and functioning of the human central nervous system (Harada, 1995; Grandjean et al., 1997). Methylmercury exposures to women of childbearing age are of great concern because a foetus is highly susceptible to adverse effects (CDC, 2001), even for low-dose exposure (Committee on the Toxicological Effects of Methylmercury, 2000). Methylmercury inhibits the growth of the foetal brain and the migration of neurons from the embryological generation layer to the final destination in the brain. This causes microcephalus in severe cases, and lower exposures result in behavioral changes and reduced cognitive and motor abilities. Methylmercury interferes with microtubule formation, cell division, and neural protein synthesis, all of which could explain the effects described

above (WHO, 1990). Epidemiological studies have therefore applied neurobehavioral tests as outcome parameters. Using domain-related neuropsychological tests in the Faroes study, a doubling of prenatal methylmercury exposure was associated with a delay in development of 1-2 months at age 7 years. This delay corresponded to about 10% of the standard deviation of the test results. Thus, the children were generally healthy, subtle neurodevelopmental effects were associated with increased mercury exposures (Grandjean et al., 1997).

There is also evidence that exposure to MeHg can have adverse effects on the cardiovascular system (blood-pressure regulation, heart-rate variability, and heart disease) even below MeHg exposure levels, which were found to be associated with neurodevelopmental effects (Committee on the Toxicological Effects of Methylmercury, 2000). Regarding cardiovascular toxicity at low-level methylmercury exposures, a Finnish population study showed that increased exposure from fish constituted a risk factor for cardiovascular mortality in adults (Ashby et al., 1990). Children with mercury poisoning often have increased heart rate and blood pressure (Sørensen et al., 1999).

The toxicological effects of methylmercury can, therefore, be summarized thus: carcinogenicity, genotoxicity, immunotoxicity, negative effects on the reproductive system, renal toxicity, cardiovascular effects, and neurotoxicity with a specific emphasis on the developing central nervous system.

To further evaluate the effects of prenatal exposure of the foetus to mixture of marine pollutants, a study was initiated in the Faroe Islands. As study design a prospective longitudinal cohort study was chosen.

Samples were collected of maternal serum at week 34 of pregnancy (last antenatal consultation at the hospital), umbilical cord blood and serum, maternal hair at parturition, and breast milk on days 3-5 post-parturition (prior to mother and child were being discharged from hospital). Nutritional habits were recorded by questionnaire (number of whale meat dinners per month during pregnancy and before pregnancy; number of fish dinners per week; ingestion of blubber with whale meat or fish). The infants were examined neurologically at about 2 weeks of age. The results of the methyl mercury levels in maternal hair and the determination of organochlorines levels in breast milk are presented. These values, which are known to correlate very well with the concentration of the pollutants in maternal and cord blood, are presented below. The effects of the pollutants on the neurological status of the infants and on the infants' on the anthropometric data are analyzed.

The purposes of the study are:

1. to document the current Faroese level of prenatal exposure of chemical substances originating from marine pollution, i.e. methylmercury, organochlorines, and hormone disrupters;
2. to relate the exposure to the mothers' dietary history;
3. to compare the exposure to marine pollutants with the exposure data, available from the cohort of 1,022 newborns in 1986-87 and estimate whether the exposure has changed;

4. to investigate whether prenatal exposure to these pollutants is related to potentially harmful effects on health, especially the function of the central nervous system and malformations of the sexual organs; and
5. to establish a birth cohort with well-documented exposure to environmental pollutants, which can be examined later on for delayed negative effects of environmental pollution on health.

6.2.2 Methods

6.2.2.1 Subject recruitment

Women who intended to deliver at the Central Hospital in Tórshavn were invited in the last trimester of pregnancy by letter to participate in the project. If they agreed, around week 34 of pregnancy (co-inciding with the last antenatal consultation at the hospital) a 50 ml blood sample was collected by phlebotomy for determination of organochlorine concentration and estrogen-like substances. In connection with the delivery, cord blood and serum, and a sample of maternal hair (100 mg) were taken. Before mother and child were discharged from the maternity ward (between day 3 to 5) a sample of breast milk was collected. Blood and milk samples were stored at minus 80° C. At approximately two weeks of age (adjusted for complete 40 weeks of gestation), the infants were examined according to the neurological optimality schedule (Prechtl, 1977). At that time all relevant obstetric data, information about smoking and alcohol consumption, as well as important socioeconomic data about the mother were recorded. A second milk sample was collected where possible. In the second half of the cohort, nutritional habits were recorded on a questionnaire filled in by a project assistant when the mother came for the neurological examination of the infant. Birth weight of the infant was recorded by the midwives. Weight at the neurological examination was measured by the examining physician on electronic baby scales, corrected to the nearest 10 grams. The infant's length was measured on a length board to the nearest 5 mm, and its head circumference was measured using a standard tape, corrected to the nearest mm. The Ethical Committee of the Faroe Islands approved the study protocol. Written informed consent was obtained from each mother.

6.2.2.2 Dietary questionnaire

The dietary questions included those previously used about the number of whale meat dinners and dinners with blubber per month during pregnancy, and before pregnancy, but we also recorded the estimated portion weight per meal, and number of other occasions where dried blubber or whale meat was eaten. The number of fish dinners per week, and the number of fulmar (*Fulmarus glacialis*) and other sea birds consumed per month, as well as the consumption of other meat (chicken and livestock) were recorded. To determine the awareness of possible hazards associated with whale meat, blubber and seabirds, the mother was asked whether the consumption of the respective food items remained the same during pregnancy, was reduced / increased somewhat or much. For whale meat and blubber, the estimated intake during the whole pregnancy was computed by multiplying the number of meals per month by 9 and then by the average weight of the portions consumed.

6.2.2.3 *Exposure variables*

6.2.2.3.1 MeHg in maternal hair

For mercury analysis, a hair sample of at least 100 mg (total thickness equivalent to a match stick; total length at least 6 cm) was cut with a pair of scissors close to the root from the occipital area; the hair was tied with a cotton string and saved in a small, marked envelope. Taking in account the delay before mercury appears beyond the hair root and the growth rate of scalp hair, a hair sample of this length will mainly represent the exposure during the first and second trimesters of pregnancy (Grandjean et al., 1999).

Mercury analysis took place at the Trace Element Laboratory, Odense University, using a Perkin-Elmer atomic absorption spectrometer 5100 with a fluid injection system. A volume of 250 µl of digested sample is then transferred to another Minisorp tube, and 2 ml of saturated KMnO₄ (Merck) in 3% H₂SO₄ (Merck) is added. The tubes are sealed with perforated parafilm (American Can Company, Greenwich, CT) and agitated before incubation in a 75° C warm water bath for 30 min. After cooling, excess potassium permanganate is reduced by careful addition of 250 µl saturated hydroxylamine hydrochloride (Merck). The solution is agitated carefully until clear. Each digested sample is prepared and analyzed in duplicate. Mercury results in µg may be converted to nmol by multiplying by 5.0.

6.2.2.3.2 Organochlorines, pesticides, and musk xylene in breast milk

6.2.2.3.2.1 *Collection, storage, and transport of samples*

While the mother was still at the hospital, a sample of transitional milk was collected directly into acid-rinsed 250 ml polyethylene bottles (Kartell, Einasco, Milano, Italy) and then frozen at -80° C.

6.2.2.3.2.2 *Laboratory procedure for organochlorines*

Milk analysis was performed in the Institute of Environmental Toxicology, Flintbek/Kiel, Germany (Dr. Heinzow) for hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), dichlorodiphenyltrichloroethane (4,4'-DDT), dichlorodiphenyldichloroethene (4,4'-DDE), trans-nonachlor, musk xylene, and polychlorinated biphenyl congeners (IUPAC number 105, 118, 138, 153, 156, and 180). The sum of the levels of PCB-138, 153, and 180 makes up about 50% of the total PCB congener mixture consumed by Faroese people. Thus ΣPCB was calculated as twice the sum of the congeners 138, 153, and 180. Samples were analyzed by gas chromatography as perviously described (Brock et al., 1996). Aldrine and Mirex were used as internal standards and added to 4 ml of a thoroughly mixed aliquot of the milk sample. Following solid-liquid-phase extraction with Florisil (Merck) and iso-hexane and elution with iso-hexane / dichloromethane (80 / 20, v / v), the eluate was evaporated with a vacuum-rotation evaporator just to dryness. The residues were reconstituted in a final volume of 2 ml iso-hexane and transferred to an autosampler vial. Analyses were performed by high-resolution gas chromatography on a Varian 3400 gaschromatograph equipped with an autosampler, a 30m DB-5 capillary column and an electron capture detector. The specific congeners were identified and quantified by comparison with response and retention time of calibration standards for each analyte of interest. At a fat content of 3% the detection limits were 0.003 µg/g lipid for DDT, 0.006 µg/g lipid for musk xylene, and 0.005 µg/g lipid for the different PCB-congeners and the other substances. For each 8 analyte-runs, one blind sample and one pool-sample were included for internal quality control. The lipid content of the samples was determined by means of the photometric Merckotest 3221 (Merck) for total lipids. Values below the detection limit were substituted by 0.0015 µg/g lipid.

6.2.2.4 *Neurological Optimality Score (NOS)*

At an age of approximately two weeks (adjusted for a gestational age of 40 complete weeks) the infants underwent a neurological examination. The Groningen Neonatal Neurological Examination systematically records major indicators of neurological function, such as posture, motility, general, muscle tone, responses, tendon reflexes, other reactions, reactivity, and stability of behavioral status during the examination. The technique for the examination of the children is a comprehensive age-specific examination as described by Prechtl (Prechtl, 1977). It has proven to be predictive of later major and minor neurological dysfunction. The neurological findings were summarized into a clinical diagnosis (normal, suspect and abnormal). A neurological optimality score (NOS) is generated, defined as the number of optimal items from a list of 60 items which were considered to be representative of the neurological condition (Touwten et al., 1980). Furthermore, two subscores were calculated according to the literature (Huisman et al., 1995), one for muscle tone (postural tone cluster score with a maximum number of 17 points) and one for reflexes (reflex cluster score, maximum: 22 points).

6.2.2.5 *Confounders / covariates*

Many factors are known to have an impact on growth and neurological development. Their effects have to be taken into account when analyzing the changes of anthropometric data, gestational age, neurological outcome, and others that are suspected to be caused by contaminants in food.

In the preliminary analyses (bivariate correlation) several different groups of confounders and covariates could be identified (see also table 6.2.1):

- factors in connection with the neurological examination, such as the identity of the examiner, the date of the examination (higher NOS toward the end of the examination period), time of examination (better scores for infants examined later in the day), and the interval between last meal and examination (lower scores seen too soon after a meal);
- gender, gestational age, age at neurological examination;
- condition during and after birth (for example Apgar score, transfer to special care unit, neonatal icterus)
- maternal- / paternal factors (educational status of parents, profession- / occupation of parents, consumption of nicotine and- /- or alcohol during pregnancy; cf. 6.2.2.5.2 OOS)

TABLE 6.2.1 POSSIBLE CONFOUNDERS FOR NOS, FOR THE WHOLE COHORT AND FOR SUBCOHORT

Confounder / covariate	Pearson (P), or Spearman correlation coefficient (S), or T-test (as applicable)		P value (two-tailed)	
	All children (N=500)	Subcohort B (N=233)	All children (N=500)	Subcohort B (N=233)
Duration of gestation (in days)	(P) 0.004	(P) 0.008	0.921	0.908
Birth weight (in g)	(P) 0.025	(P) -0.080	0.575	0.222
Percentile of birth weight*	(S) 0.026	(S) -0.144	0.565	0.027
Corrected age at NOS (in days)	(P) -0.060	(P) -0.108	0.180	0.099
Apgar 5 minutes	(S) 0.042	(S) 0.033	0.345	0.616
Neonatal illness (no / yes)*	(T) -0.738	(T) 0.102	0.461	0.919
Head circumference (OFC) at NOS	(P) -0.067	(P) -0.120	0.135	0.068
Percentile of OFC at NOS*	(S) -0.113	(S) -0.147	0.012	0.025
Body length at NOS	(P) 0.083	(P) -0.054	0.065	0.414
Percentile of body length at NOS*	(S) -0.114	(S) -0.147	0.011	0.025
Icterus at NOS (no / yes)	(T) 1.569	(T) 0.491	0.117	0.624
Examiner (A / B)	(T) 9.473	---	<0.001	---
Date of examination	(P) 0.159	(P) 0.151	<0.001	0.022
Time (during day) of examination	(P) 0.080	(P) 0.083	0.081	0.215
Interval between meal and examination	(P) 0.244	(P) 0.135	<0.001	0.045
Stability of states (no / yes)*	(T) -3.902	(T) -1.962	<0.001	0.090
Maternal age	(P) 0.029	(P) -0.140	0.513	0.033
BMI	(S) -0.058	(S) -0.161	0.193	0.014
Smoking of mother during pregnancy*	(S) -0.070	(S) 0.005	0.118	0.938
Alcohol of mother during pregnancy*	(S) -0.075	(S) -0.050	0.093	0.448
Education of mother*	(S) -0.005	(S) 0.025	0.912	0.705
Education of father*	(S) -0.023	(S) -0.013	0.615	0.841
Level of father's / parents' occupation and profession*	¹ (T) 1.906 ² (T) 2.386	(T) 2.667 (T) 3.524	0.057 0.017	0.008 0.001
Parity	(S) 0.060	(S) -0.111	0.184	0.090
Early spontaneous abortions (no / yes)	(T) 1.888	(T) 1.437	0.061	0.152
Other non-obstetrical diseases during pregnancy (no / yes)*	(T) -2.199	(T) -4.380	0.034	0.003
Obstetric Optimality Score	(P) -0.063	(P) 0.015	0.156	0.853

* For further details: see text

1 : T-value resp. significance level for correlation with father's occupation / profession

2 : T-value resp. significance level for correlation with highest parents' occupation / profession

Explanations:

- percentile of birth weight:

0 <2.3%
 1 2.3%-5%
 2 6-10%
 3 11-50%
 4 51-90%
 5 91-95%
 6 >95%

- percentile of OFC (head circumference) and body length at NOS:

0 <10%
 1 10-50%
 2 51-90%

3 >90%

- neonatal illness (information was taken from the Obstetric Optimality Score):
 - 0 no neonatal illness
 - 1 any kind of neonatal illness (feeding problems, infection, neonatal icterus, problems after birth requiring observation)
- stability of states:
 - 0 behavioral status during neurological examination stable
 - 1 behavioral status during neurological examination unstable
- smoking of mother (partner) during pregnancy (see 6.2.2.5.4)
- alcohol consumption of mother during pregnancy (see 6.2.2.5.3)
- education of mother / father (see 6.2.2.5.5)
- father's / parents' occupation and profession (low / high) (see 6.2.2.5.5)
- other non-obstetric conditions during pregnancy: information was taken from the questionnaire for the Obstetric Optimality Score (OOS).

6.2.2.5.1 Examiner

There were three different examiners involved in the examination: US functioned as supervisor in the beginning of the study and therefore examined only a few children. Two experienced paediatricians A and B performed the examinations. The scoring of the neurological examination is known from the experience of other study groups to vary somewhat between examiners as individual judgment must be exerted, when performance of the infant is borderline. Therefore the dependence of the result of the neurological examination in form of the NOS of the two main examiners was tested. The children seen by examiner A reached a significantly higher score than the rest of the group (see 6.2.3.2.1). When ever necessary and applicable, the data analysis was done for the combined group as well as for the two subcohorts A and B.

6.2.2.5.2 Obstetric Optimality Score (OOS)

Obstetric data were evaluated according to the obstetric optimality list, as described by Touwen (Touwen et al., 1980). The list used in this study consisted of 73 items that measure socioeconomic status and pre-, intra-, and immediate post-partum conditions (for a complete list and criteria for optimality see Table 6.2.21 in the appendix). The items were completed using information recorded in the hospital records as well as that given verbally by the mother. For several items (such as marital status at time of delivery, participation in parenthood course, education of mother's father) no information was available. By counting the number of items that fulfilled the preset criteria for optimality (Prechtl, 1980), the Obstetric Optimality Score (OOS) was calculated.

6.2.2.5.3 Alcohol during pregnancy

Two separate analyses were done to examine the influence of maternal alcohol consumption on the growth and the neurological outcome of the infant. Two

different scoring lists were used: dichotomized as “no alcohol at all” versus “any amount of alcohol during pregnancy” and an ordinal variable. The possible answers were:

- 0 no alcohol at all
- 1 alcohol less than once per month
- 2 alcohol about once per month
- 3 alcohol few times per month
- 4 alcohol about once per week
- 5 alcohol 2 to 3 times per week
- 6 alcohol 4 to 5 times per week
- 7 alcohol nearly every day

Where necessary (e.g. for linear regression model) the information about alcohol was dichotomized into “no alcohol during pregnancy” and “yes, alcohol during pregnancy”.

6.2.2.5.4 Smoking of mother and / or partner

For the registration of smoking habits of mother or mother's partner during pregnancy five categories were used:

- 0 no smoking at all
- 1 1-5 cigarettes / day
- 2 6-10 cigarettes / day
- 3 11-15 cigarettes / day
- 4 16-20 cigarettes / day
- 5 >20 cigarettes / day

As for “alcohol during pregnancy”, the information about smoking was also dichotomized into “no smoking during pregnancy” and “yes, smoking during pregnancy”.

6.2.2.5.5 Education / profession / occupation of parents

Education of parents was categorized into:

- 0 no elementary school examination (yet), i.e. less than nine years of schooling
- 1 nine years of schooling
- 2 ten years of schooling including secondary school (HF-prógv)
- 3 12 years of schooling (FHS)
- 4 13 years of schooling with university entrance qualification (studentsprógv / hægri handelsskúlaprógv)

Scoring of profession / occupation of parents which was done by the Faroese staff using these classes:

- 0 under training
- 1 unskilled worker
- 2 short theoretical education / training
- 3 apprenticeship finished
- 4 middle long theoretical education
- 5 long theoretical education

To calculate certain correlations, the higher of the two scores of the education of mother and father were chosen. To obtain a dichotomized variable, education was divided into the following two groups:

- 0 only basic school exam (max. 10 years in school)
- 1 more than basic school exam (more than 10 years in school).

For profession and / or occupation, a dichotomized variable was used with the following subdivision:

- 0 score for mother's / father's occupation / profession was 0-3
- 1 score for mother's / father's occupation / profession was 4 or 5

6.2.2.6 *Statistical methods*

All statistical analyses were performed using the Statistical Package for the Social Sciences, version 10.0 (SPSS for Windows 10.0).

Quantitative data are described by their arithmetic means, and the standard deviation. In some cases the 10th, the 90th percentile and the range are also included. For variables with skewed distribution such as contractions of exposure data, maternal body mass index (BMI), and most dietary variables, geometrical means with spread factor (geometric standard deviation) were computed to normalize the distribution and to obtain a better statistical fit. For these data log 10-transformed values were used in parametric tests. Percentage values are used to describe the distribution of qualitative variables.

Mean differences in group comparisons were calculated by using the T-test for independent samples. For proportions chi-square analysis was used to determine differences between groups.

Bivariate correlations between variables were tested by calculating the Pearson correlation coefficient in normally distributed data, and Spearman's rho for rank orders. The statistical significance of correlations was determined by computing the p-values for the coefficients. For the correlation of several variables it became necessary to calculate partial correlation coefficients while controlling for major covariates (e.g. sex in the correlation between exposure data and NOS). For the sake of uniformity, only two-tailed significance levels were used.

Linear regression analyses were carried out for the main outcome variables birth weight and NOS. Items were chosen as covariates and tested if they were known as confounders from other studies or if it appeared plausible that they might function as confounders.

6.2.3 **Results**

6.2.3.1 *Description of subjects*

During the period of cohort formation a total of 1,152 children were born alive in the National Hospital in Tórshavn. These included one set of triplets and 16 pairs of twins, thus leaving 1,117 singletons, (7 pairs of twin were examined but not included in the cohort). In 1,019 births, the mother lived in the primary catchment area of the National Hospital (i.e. not the northern or southern islands). This area was the proposed catchment area for the project. A total of 608 of the infants were examined, giving an overall participation rate of 59.7%. This rate represents an underestimate, as inclusion required,

e.g. cord blood, thereby eliminating Caesarean section and births where complications prevented the midwife from taking a blood sample. Due to prematurity, the data of 10 children were not used for analysis (4 children of 35 weeks of gestation, 6 children of 36 weeks of gestation), and 7 children were excluded because they were older than 5 weeks of corrected age at examination – an age when the used technique for the neurological examination no longer is valid. Eight infants were examined when only two days old (chronological age) when birth stress factors still influence heavily the result of the neurological examination. Therefore their data were not used for the analyses. The exclusions (for reasons other than incompleteness of exposure data) amounted to 25/608 or 4.1%. In addition, hair for mercury analysis and / or milk for analysis of organochlorines, pesticides, and musk xylene were in several cases not obtained in sufficient amount to allow chemical analysis. Thus the data of 500 mother-infant pairs remained for this preliminary report.

6.2.3.1.1 Infants: anthropometric data and other characteristics of the children in the study.

The most important information about the infants in the study cohort is displayed in the following two tables (table 6.2.2 and table 6.2.3).

Table 6.2.2 DESCRIPTION OF INFANTS

	Mean	Median	SD	Range
Birth weight (g)	3,743	3,750	473	2,450 – 5,400
Gestational age (completed weeks)	40.1	40	1.2	37 – 42
Gestational age (days)	283.2	283	8.0	260 – 300
Apgar score at 1 min	9.2	9	1.0	3 – 10
Apgar score at 5 min	9.9	10	0.4	6 – 10
Corrected age at NOS (days)	16.9	16	5.8	-1 – 34
Chronological age at NOS (days)	13.7	13	7.0	3 – 35

Table 6.2.3 NON-METRIC CHARACTERISTICS OF INFANTS

	N	Percent
Gender: male / female	267 / 233	53.4 / 46.6
Birth weight (small-for-dates / appropriate for gestational age / large-for-dates)	22 / 404 / 74	4.4 / 80.8 / 14.8
Head circumference at neurological examination: appropriate for age (small / appropriate for age / large) N = 499	6 / 385 / 108	1.2 / 77.2 / 21.6
Body length at neurological examination appropriate for age (small / appropriate for age / large) N = 498	6 / 385 / 107	1.2 / 77.3 / 21.5

N = Number of infants where data are available

It is known that Faroese children are very large at birth (Olsen et al., 1985), but no specific growth charts for Faroese children are available. Therefore German (for birth weight) and British (for length and head circumference) charts were used as reference anthropometric data for the Faroese infants. The fact that only term-infants were included in the cohort explains why in

the study cohort only 4.4% small-for-gestational-age children (by definition those who constituted the 10 lightest percent of a birth cohort), but 14.8% of large-for-date infants (instead of 10%) were found. The percentage of children with a head circumference or length above the 90th percentile was even higher.

Some more significant differences were identified between boys and girls in the study cohort:

Body temperature at the neurological examination was higher in boys than in girls (p-value: 0.014); the same was true for maternal age (p-value: 0.046) and parity (p-value: 0.024). The OOS was also slightly higher in girls (p-value: 0.062). The time between rupture of membranes and delivery was longer in boys (0.047). These observations may represent spurious findings, but possible links to endocrine disruption cannot be ruled out.

Clinical examination of the 267 boys revealed one boy with a unilateral undescended testis, for two boys the information about the testicles was missing. As an ultrasound examination was not carried out, the true number of cases may have been underestimated. Two children had a mild hypospadias. The concentration of all PCB-congeners, DDE, and DDT were in the lower range of the general distribution. For the small number of cases no statistical analyses were done.

Estimation of gestational age was based on the mother's report of her last menstrual period. If the date of the last menstrual period had been unclear, then the expected date of delivery calculated by ultrasound examination of the foetus at approximately week 22 of pregnancy was used. The duration of a complete pregnancy was taken to be 280 days or 40 weeks. Prolongation or shortening of the period was expressed in days as well as in completed weeks of gestation.

6.2.3.1.2 Background data about the mothers and their partners

Of major interest is all information about the possible covariates of neurological outcome of the child and its growth that are associated with the family background data: maternal age, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, maternal medical history, and socioeconomic status. The distribution of maternal pre-pregnancy body mass index [weight in kg / (length in m)²] was skewed. Therefore BMI was log 10-transformed before using it in parametric analyses. For a summary of characteristics of the parents, see table 6.2.4 and table 6.2.5.

TABLE 6.2.4 CHARACTERISTICS OF THE PARENTS, PART I (METRIC DATA)

	Mean	Median	SD	Range
Mother's age (in years)	29.1	30	5.1	17 – 43
Mother's weight (in kg)	64.4	63	10.3	43 – 106
Maternal height (in cm)	165.3	165	5.8	148 – 184
Body mass index BMI (in kg / m ²)*	23.3	22.9	1.2	16.6 – 39.6
Weight gain during pregnancy	15.0	15.0	4.9	-2 – 32
Parity (number of previous pregnancies)	1.3	1	1.1	0 – 8
Obstetric Optimality Score (out of 73)	62.2	63	3.9	49 – 72

*: For skewed distribution of body mass index, geometric mean and spread factor are given in the table instead of arithmetic mean and standard deviation

TABLE 6.2.5: CHARACTERISTICS OF THE PARENTS, PART II (ORDINAL DATA)

	N	Percentage
BMI of mother (low / optimal = 18.8 - 24.2 / high)	500	4.8 / 61.0 / 34.2
Alcohol during pregnancy (none / less than once or once per month / more frequently)	500	58.0 / 35.6 / 6.4
Smoking of mother (no / yes)	500	71.2 / 28.8
Smoking of father (no / yes)	497	61.4 / 38.6
Parity (0 / 1 / 2 / >2)	500	26.6 / 35.0 / 25.6 / 13.8
Mother's nationality (Faroese / Danish / other)	500	96.4 / 2.0 / 1.6
Father's nationality (Faroese / Danish / other)	498	96.2 / 2.8 / 1.0
Basic school education of mother ¹	500	1.0 / 1.4 / 13.0 / 84.6
Higher school education of mother ²	500	27.4 / 34.4 / 19.8 / 18.4
Occupation / profession of mother ³	500	8.6 / 28.0 / 9.4 / 27.0 / 22.2 / 4.8
Basic school education of father ¹	492	9.6 / 2.0 / 13.2 / 75.2
Higher school education of father ²	493	67.3 / 21.5 / 4.7 / 6.5
Occupation / profession of father ³	495	5.1 / 10.3 / 3.2 / 42.2 / 29.3 / 9.9

1: 7 years / 8 years / 9 years / 10 years or more

2: None / high school diploma / high school equivalent / commercial school (1 year)

3: Scoring of occupation of parents: no professional education completed or under training / unskilled worker / skilled worker / medium long training / longer training / university training; scoring of profession of parents: under education / unskilled worker / lower employee / middle class employee / independent middle class occupation / higher class occupation; for the item "occupation / profession of mother or father" the highest one of all four variables was selected

6.2.3.1.3 Possible selection bias in study cohort: Comparison of participating and non-participating mother-infant pairs

In the study period (1.1.1998 – 29.2.2000), 1,152 children were born alive in the National hospital. Infants who were born at the hospitals in Klaksvík or Tvøroyri and those, who were born in Tórshavn, but were living with their mothers on the northern islands (Borðoy, Viðoy, Kunoy, Karlsoy, Fugloy, and Svínay) or on the southern island (Suðuroy) were not included in the cohort. However, one child from Klaksvík and one child from Sandvík (Suðuroy) were found to be misclassified. After exclusion of premature infants of less than 37 weeks of gestation, 1,074 singletons remained. To rule out selection bias for the children in the cohort, those infants of the eligible 1,074, who did not participate, were compared with those of the cohort examined (table 6.2.6). Information was obtained from the birth records that are used for official statistics. Children born by caesarian section or vacuum extraction (instrumental delivery) are underrepresented in the cohort due to the practical difficulties of collecting blood and hair samples under such circumstances.

As major differences between participants and non-participating mother-infant pair, maternal age and parity (younger mothers and those with less children) participated less often. The ratio of infants who had to be transferred to the neonatal intensive care unit was higher in the study group. The number of non-smoking mother is slightly higher in the study group. Although statistically significant, the differences were small and possibly of limited importance for the study.

TABLE 6.2.6 COMPARISON OF PARTICIPANTS AND NON-PARTICIPANTS

	Cohort of 500 children	Non-participants (N=483)	Significance
Sex (male / female)	267 / 233 (53.4% / 46.6%)	254 / 229 (52.6% / 47.4%)	N.s.
Birth weight (g)	3743 / SD 473	3686 / SD 478	N.s.
Length (cm)	53.4 / SD 2.1 (N=499)	53.2 / SD 2.2 (N=481)	N.s.
Gestational age (weeks)	40.1 / SD 1.2	40.1 / SD 1.3	N.s.
Apgar at 1 min.	9.2 / SD 1.0	9.1 / SD 1.1 (N=482)	N.s.
Apgar at 5 min.	9.9 / SD 0.5	9.9 / SD 0.4 (N=482)	N.s.
Pediatrician present at delivery (no / yes)	93.2% / 6.8%	93.6% / 6.4%	N.s.
Transfer to intensive care or observation (no / yes)	97.6% / 2.4% (N=497)	94.6% / 5.4% (N=482)	0.016
Maternal age (years)	29.0 / SD 5.2	28.3 / SD 5.7	0.038
Parity (including this one)	2.3 / SD 1.1	2.1 / SD 1.1 (N=482)	0.018
Civil status of mother (married or living together with father of child / other)	92.4% / 7.6% (N=397)	91.6% / 8.4 (N=370)	N.s.
Smoking of mother (no / yes)	75.4% / 24.6% (N=491)	69.5% / 30.5% (N=469)	0.043
Complications during pregnancy (no / yes)	89.6% / 10.4%	85.5% / 14.5%	N.s.
Induction of labor (no / yes)	73.2% / 26.8%	71.2% / 28.8%	N.s.
Presentation at birth (vertex / other)	87.4% / 12.6%	86.3% / 13.7%	N.s.
Instrumental delivery (no / yes)	87.4% / 12.6%	85.1% / 14.9%	N.s.
Analgesia during delivery (none, acupuncture / more)	48.2% / 51.8%	46.0% / 54.0%	N.s.
Complications during delivery (no / yes)	88.4% / 11.6%	84.5% / 15.5%	N.s.
Color of amniotic fluid (clear / not clear)	88.6% / 11.4% (N=491)	88.2% / 11.8% (N=473)	N.s.

N.s.: Not significant (2-tailed $P > 0.05$)

If information was not available for all children, the number of cases is given in parenthesis.

For ordinal parameters mean and standard deviation (SD) are given.

According to the study protocol, only mothers, who reside in the Streymoy, Eysturoy, Vágar, and Sandoy, were invited to participate. Therefore only very few participants came from the other islands: one child from the southern islands (Suðuroy), one from the northern islands. These two children were subsequently excluded. Children from Eysturoy are over-represented in the study cohort, while those from Vágar participated significantly less than expected, possibly due to longer travel distances.

TABLE 6.2.7 MOTHER'S RESIDENCE

Mother's residence	Study cohort (N=500)	Non-participants (N=483)	Significance
Tórshavn ¹	51.0%	51.4%	N.s.
Streymoy ² outside of Tórshavn area	15.4%	14.1%	N.s.
Vágar	3.6%	8.9%	0.001
Eysturoy	27.4%	21.7%	0.039
Sandoy	2.6%	3.9%	N.s.

1: Tórshavn, Argir, Hoyvík, Kaldbak

2: Vestmanna, Kvívík, Leynar, Nólsoy, Velbastaður, Kollafjørður, Hovvík, Hvalvík, Saksun, Haldarvík, Eiði, Oyrabakk, Streymnes, Koltur, Hestur

6.2.3.2 Covariates / confounders

6.2.3.2.1 Examiner

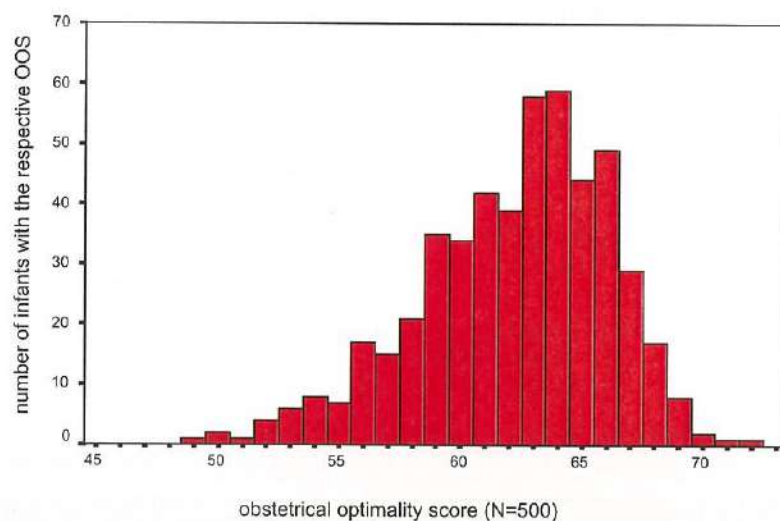
Three examiners were involved in the study: A, B and C. Only few examinations were done by C (5/500), A saw 262 and B 233 of the children. In order to correct for inter-observer differences, the examiner was included as an explanatory variable. The two subcohorts that were examined by pediatrician A and pediatrician B are analyzed here to look for substantial differences between the groups. All examiners were unaware of the exposure to harmful contaminant.

No significant objective differences could be identified between the two subcohorts. Comparison of the exposure data of the two subcohorts revealed no significant differences. However, some differences in NOS scores were seen (see section 6.2.3.7.1).

6.2.3.2.2 OOS (Obstetrical optimality score)

The mean of the OOS was 62.2 (out of 73) with a standard deviation of 3.9, the results for the OOS ranged from 49 to 72 points. The quartiles (P25, P50, and P75) were calculated to 60, 63, and 65 points.

FIGURE 6.2.1: DISTRIBUTION OF OOS



A higher OOS was achieved with prolongation of gestation (p-value for Pearson correlation: 0.036). Boys showed a tendency to lower OOS compared with girls (means in boys: 61.9, girls: 62.6; 2-tailed significance 0.062). Birth weight did not influence the OOS in this cohort.

Lower OOS (more risks and less favorable conditions during pregnancy and delivery) are connected with increasing maternal age, increasing parity, increasing weight of gravida, higher BMI, higher weight gain during pregnancy, and if mother and / or partner are smokers. Higher school education of father and / or mother (but not with profession or occupation of parents) and greater height of mother lead to a higher OOS. The magnitude of correlations reaching significance level between parental characteristics and OOS are shown in table 6.2.8.

TABLE 6.2.8 SIGNIFICANT CORRELATIONS BETWEEN PARENTS' CHARACTERISTICS AND OOS

	Correlation coefficient (P=Pearson, S=Spearman)	P-value (2-tailed)
Maternal age	-0.202 (P)	<0.001
Weight of mother	-0.205 (P)	<0.001
Height of mother	0.110 (P)	0.014
Body mass index of mother (weight / height ²)	-0.250 (S)	<0.001
Weight gain during pregnancy	-0.089 (P)	0.046
Smoking of mother*	-0.141 (S)	0.002
Smoking of partner*	-0.188 (S)	<0.001
Parity	-0.159 (S)	<0.001
Mother's school education*	0.199 (S)	<0.001
Father's / partner's school education*	0.200 (S)	<0.001

* : For scoring: see 2.5.3, 2.5.4 resp. 2.5.5

6.2.3.2.3 Alcohol during pregnancy

The older the mother, the higher is the consumption of alcohol during pregnancy (p=0.005). With increasing height of mother, alcohol is also consumed more often. Astonishingly, a significant positive correlation was found between alcohol and gestational age (Spearman's rho 0.109, p=0.015), i.e. more alcohol leads to higher gestational age! Mean of length of gestation for teetotalers was 282 days, for children of mothers, who consumed alcohol, 284 days (level of significance for t-value: 0.011). However, consumption of alcohol during pregnancy is correlated with a higher score for mother's school education, occupation and profession (p<0.001 for all of them). The same is true for the father's education (p<0.001) and less so for profession (p=0.020). Parents who are not Faroese drink more alcohol (significance of result of T-test is <0.001). The amount of alcohol consumption is positively correlated with more legal abortions (p=0.001) and more late spontaneous abortions, i.e. during 16-28th gestational week (p<0.001). Note must be taken that the alcohol consumption levels in this population are quite low. On the other hand, the NOS of infants of mothers who consumed alcohol during pregnancy is lower, but not significantly different to that of children of teetotaler mothers (p-value for Spearman's rho: 0.093). When analyzing some of the specific items of the NOS, it was shown that tremor intensity and tremor amplitude increased with higher consumption of alcohol (p<0.05). Although possibly of limited importance due to the limited alcohol intake, these tendencies have to be kept in mind in further analyses of the data.

6.2.3.2.4 Smoking of mother and / or partner

A clear negative correlation was seen between birth weight ($p=0.003$), body length at NOS ($p=0.019$), and OOS ($p=0.002$) with smoking of mother. As could be expected, intrauterine growth retardation in one of the previous pregnancies (Fischer's exact test: 0.008) or in the present one (Chi-square: 0.005) was highly significantly correlated to maternal smoking. A higher Apgar score at one ($p=0.007$) and five minutes ($p=0.045$) is found in children of smoking mothers, the same is true if the father is a smoker but with an even higher significance (Apgar one minute: $p=0.002$, Apgar five minutes: $p=0.029$). Maternal smoking is correlated with more overshooting movements (significance of $F<0.001$) during the neurological examination. Passive smoking of mother (i.e. partner's smoking) is significantly correlated to muscular hypertonia of the infant (significance of F : 0.023) and with the diagnosis of "opisthotonus" in the infant (0.012). The conclusion of the neurological examination was more frequently described as "suspect" if both parents were smokers (Fischer's exact test: 0.027). The NOS itself was not significantly correlated to the smoking-status of the parents. Given the known effects of maternal smoking, the prevalence of smoking in these mothers, and the types of associations identified, smoking appears to be an important covariate.

6.2.3.2.5 Education, profession, and occupation of parents

6.2.3.2.5.1 *Description*

For distribution of parental education, profession and occupation: see section 6.2.3.2.

6.2.3.2.5.2 *Effects of education, profession, and occupation of parents*

A higher score for the profession or occupation of one of the parents led to a higher birth weight and a higher percentile of birth weight as well as higher percentiles of length and head circumference at NOS. Duration of gestation and Apgar-score are unaffected by educational and / occupational status of parents. Reduced smoking of the pregnant mother and father are significantly correlated with a higher school education ($p=0.002$ / $p<0.001$), higher occupation ($p=0.017$ / $p=0.021$) and profession ($p<0.001$ / $p=0.004$). These anticipated associations support the need to consider all of these covariates together.

6.2.3.2.6 Nationality of parents

Faroese mothers are younger (significance of T-test 0.017) and have a lower pre-pregnancy body weight (0.042). The birth weight of infants of Faroese mothers as compared with infants of non-Faroese mothers is significantly higher (difference of the mean more than 230g, significance of T-test 0.002). The same is true for the percentiles of head circumference and body length at neurological examination (significance of F : 0.007 resp. 0.008). This may be partially explained by the fact that Faroese mothers smoke less than non Faroese mothers ($p=0.032$). On the other hand their partners are more frequently smokers if their nationality is not Faroese ($p=0.022$). Faroese mothers are more often teetotaler than mothers of other nationality (<0.001). The Apgar-score of their children after one minute is significantly higher (0.020).

6.2.3.3 Dietary questionnaire

In this paragraph, the result of the analyses of the 262 dietary questionnaires are presented. Only a few of the questionnaires were filled in by the mothers themselves; most were filled in by a project assistant, who questioned the

mothers, thus helping to ensure uniformity of scoring. A summary of the results is given in table 6.2.9 and table 6.2.10.

TABLE 6.2.9 DIETARY HABITS OF MOTHERS DURING PREGNANCY, PART I

	N	Mean	Median	Standard deviation	P 25	P 75	Range
Total number of meals with whale meat per month	261	1.8	1	1.9	0	3	0 – 8
Total whale meat during pregnancy (in g.)	217	326	100	438	0	600	0 – 2400
Number of meals with blubber per month	256	1.4	0	2.7	0	2	0 – 18
Total amount of blubber during pregnancy (in g.)	238	90.8	0	293	0	100	0 – 3600
Number of other meals with dried whale meat	257	0.6	0	1.9	0	1	0 – 18
Number of other meals with dried blubber	258	1.6	0	3.5	0	1	0 – 18
Number of fish meals per week	256	2.1	2	1.1	1	3	0 – 5
Number of meals with fulmar during pregnancy	258	1.2	0	2.8	0	1	0 – 27
Number of meals with other seabirds during pregnancy	257	1.7	1	4.8	0	2	0 – 36
Number of other meals with meat per week*	253	3.3	3	1.2	3	4	1 – 6

* Normal distribution, the distribution of all other variables was skewed

For those who ate whale meal during pregnancy (N=120), the geometric mean of the total amount was 427g, with a spread factor of 2.4g. For those who ate blubber (N=83), the geometric mean of the total amount of blubber, consumed during pregnancy, was 151 (spread factor 2.5). For the other data with a skewed distribution, the geometric mean of those, who ate the food item, was:

- number of other meals with dried whale meat during pregnancy 1.6 (spread factor 1.9),
- number of other meals with dried blubber during pregnancy 2.4 (spread factor 2.7),
- number of fish meals (per week) 1.8 (spread factor 1.8),
- number of meals with fulmar during pregnancy 1.9 (spread factor 2.2),
- number of meals with other seabirds during pregnancy 2.0 (spread factor 2.3), and
- total number of meals with any seabird during pregnancy 2.5 (spread factor 2.5).

6.2.3.3.1 Dietary habits and parental characteristics

Here, the correlation between parental characteristics and diet was examined as an important way to identify possible predictors of dietary habits. No significant correlation was found between mother's age, height, weight, or BMI with dietary habits during pregnancy.

Women who ate more whale meat meals during pregnancy reported abortion in late pregnancy (16-28 weeks) more often (significance of t-value of independent samples test: 0.012).

The lower the parents' education, the more whale meat and blubber were consumed. Father's education is further correlated to the number of fish meals: the higher the level of education, the less fish meals were eaten by the mother during pregnancy. In interpreting this information, it must be kept in mind that the Faroese authorities have issued advisories regarding whale consumption, and that the impact of such recommendations may be related to the subjects educational level.

The more alcohol the mother consumed during pregnancy, the more meals with dried whale meat she ate (significance of Spearman's rho: 0.019). Maternal smoking during pregnancy (dichotomized into "yes" or "no") is strongly correlated with eating blubber or whale meat on two consecutive days (2-sided significance for Fischer's exact test; 0.009 and 0.024, respectively) and a similar tendency was seen for the smoking of the partner.

Father's nationality (Faroese or not) and mother's fish consumption are significantly correlated with each other: if the father was Faroese, 2.1 fishmeals per week were reported compared to 1.9 for a non- Faroese father (2-tailed significance for independent-samples T-test: 0.010), but no significant correlation was seen with mother's nationality.

Again, these associations in general are quite in accordance with expectations and confirm the importance of considering these factors as covariates.

TABLE 6.2.10 CORRELATION BETWEEN MOTHER'S DIETARY HABITS DURING PREGNANCY OF AND PARENT'S EDUCATION / PROFESSION / OCCUPATION

Spearman's rho p-values (2-tailed significance)	Mother's education	Mother's occupation	Father's education	Father's occupation
Total amount of whale meat (N=217)	-0.199 0.003	-0.208 0.002	-0.266 <0.001	N.s.
Total amount of blubber (N=238)	-0.082 0.205	-0.184 0.004	-0.162 0.013	N.s.
Number of other meals with dried whale meat (N=257)	N.s.	0.115 0.066	N.s.	N.s.
Number of other meals with dried blubber (N=258)	0.192 0.002	N.s.	N.s.	N.s.
Number of fish meals per week (N=256)	N.s.	-0.105 0.095	-0.250 <0.001	N.s.
Number of other meals with meat per week (N=253)	N.s.	N.s.	0.122 0.054	N.s.
Change of usual consumption of blubber during pregnancy* (N=258)	N.s.	N.s.	N.s.	N.s.
Change of usual consumption of seabirds during pregnancy* (N=255)	N.s.	-0.117 0.063	N.s.	N.s.

* : Possible answers were "much less", "less", "the same", "more", and "much more"

6.2.3.3.2 Diet and present recommendations

"Women who intend to get pregnant during the next three months, women who are pregnant or those who are breastfeeding should not eat any whale meat at all. Livers and kidneys of whale should be eaten at all." The health authorities of the Faroes issued this advisory in 1998 (Heilsufrøðiliga Starvsstovan, 1998). The results of the questionnaires, all of which were completed after 1 January 1999, revealed that many mothers had either been unaware of the recommendations or had ignored them. More than 60% of women ate whale meat, and about 30% consumed blubber during pregnancy.

No difference could be seen between the number of mothers who refrained from whale meat or blubber meals in the first months of 1999 and in the last three months of the study (i.e. December 1999 and February 2000).

How many mothers reduced their consumption of whale during pregnancy? 71% of the mothers stated that they reduced the consumption of whale meat during pregnancy; 61% ate less blubber. Only very few of them increased the amount of whale meat, and no one ate more blubber during pregnancy than before. About 65% of the mothers who filled the questionnaire had eaten at least one meal containing seabirds; 20% had eaten fulmar for dinner on more than one occasion; 28% had eaten other species at least twice during pregnancy. These results suggest that the advisory has had a substantial effect, though it was by no means fully successful.

TABLE 6.2.11 DIETARY HABITS OF MOTHERS DURING PREGNANCY, PART II

	N	Number; percentage
No whale meat at all	261	97; 37.2%
No blubber at all	256	155; 60.5%
Consumption of whale meat during pregnancy: less / unchanged / more	259	184 / 74 / 1 71.0% / 28.6% / 0.4%
Consumption of blubber during pregnancy: less / unchanged / more	258	158 / 100 / 0 61.2% / 38.8% / 0%
No fulmar at all	258	153; 59.7%
No other seabirds at all	257	128; 49.8%
No seabirds at all	256	89; 34.8%
Consumption of seabirds during pregnancy: Less / unchanged / more	255	50 / 203 / 2 19.6% / 79.6% / 0.8%

6.2.3.4 Exposure Variables

For their skewed distribution, the geometric mean and spread factor were calculated to characterize the concentration of exposure variables in maternal hair and breast milk. Very wide ranges of exposure data were found. The ratio of the highest to the lowest concentration of the substances are given in the column "max. / min." in table 6.2.12.

TABLE 6.2.12 MERCURY ($\mu\text{G/G}$ IN MATERNAL HAIR) AND POPs ($\mu\text{G/G}$ LIPID IN BREAST MILK)

	Mean	Geometric mean	Spread factor	P25	P75	Min.	Max.	Max. /min.
Mercury	2.998	2.073	2.460	1.190	3.898	0.0200	32.72	1636
HCB	0.044	0.039	1.673	0.026	0.052	0.0090	0.256	28
β -HCH	0.023	0.020	1.623	0.015	0.027	0.0015	0.210	140
PCB-105	0.018	0.012	2.623	0.007	0.022	0.0015	0.109	73
PCB-118	0.082	0.059	2.280	0.037	0.105	0.0015	0.629	419
PCB-138	0.280	0.215	2.038	0.137	0.344	0.0150	3.147	210
PCB-153	0.372	0.287	2.019	0.183	0.460	0.0190	3.830	202
PCB-156	0.027	0.021	2.069	0.014	0.035	0.0015	0.164	109
PCB-180	0.208	0.160	2.058	0.100	0.262	0.0070	1.817	260
Σ PCB*	1.720	1.330	2.022	0.848	2.078	0.0820	17.59	215
4,4'-DDT	0.035	0.022	2.731	0.013	0.043	0.0015	0.482	321
4,4'-DDE	0.857	0.599	2.280	0.359	0.991	0.0590	11.35	192
Sum DDT+DDE	0.892	0.624	2.282	0.376	1.036	0.0610	11.83	194
Ratio DDT/DDE	0.042	0.037	1.662	0.028	0.053	0.0025	0.117	47
Trans-nonachlor	0.153	0.099	2.592	0.056	0.192	0.0050	1.889	378
Musk xylene	0.016	0.013	1.867	0.008	0.017	0.0015	0.297	198

*: 2.0 x PCB (138+153+180)

6.2.3.4.1 Mercury

The mean concentration of mercury in maternal hair was calculated to nearly 3 µg/g of hair, the geometric mean for the skewed distribution reaching only 2 µg/g. Ten hair samples (5%) exceeded the value of 10 µg/g MeHg, a limit previously thought to protect against clinical adverse effects. In 74 (14.8%) samples more than 5 µg/g was found. The range of the distribution was wide: the lowest samples containing not more than 0.02 µg/g, the one with the highest load nearly reaching 33 µg/g. Thus the ratio between the highest and the lowest concentration calculates to more than 1,600 (see table 6.2.12).

6.2.3.4.2 PCB / POPs

The few samples containing non-detectable levels of one of the POPs were given the concentration of 0.0015 µg/g for purposes of statistical analyses. This level corresponds to a concentration below the detection limit. For HCH, one sample was below detection limit; for PCB-105, 36 samples; for PCB-118, one; for PCB-156, seven; 4,4'-DDT, 16, and musk xylene, one.

The mean for the ΣPCB (twice the sum of PCB's 138, 153, and 180) was 1.7 µg/g lipid, and the geometric mean 1.3 µg/g lipid; 93 milk samples (18.6%) had a ΣPCB >2.5 µg/g. There were 10 samples being above a 6 µg/g limit

To get an impression of the extent of contamination of the breast milk of the Faroese mothers with persistent organic pollutants, the concentrations of the present cohort were compared to the reference values given by the German Federal Agency for the Environment (Institut für Wasser-, Boden und Lufthygiene, 1999) These values represent the 95th percentile of the concentrations found in unselected breast milk sample in 1994 (N=1757). The limits for the two di-ortho congeners IUPAC number 180 and 153 were exceeded by more than one-third and nearly half of the Faroese samples, respectively (table 6.2.13).

TABLE 6.2.13 CONCENTRATIONS OF POPs IN BREAST MILK AND GERMAN REFERENCE VALUES

	German reference limit for breast milk samples	Number of samples exceeding this limit	Percentage of samples exceeding the limit
β-HCH	0.1 µg/g lipid	3	0.6%
HCB	0.3 µg/g lipid	0	0%
PCB-138	0.3 µg/g lipid	2	0.4%
PCB-153	0.3 µg/g lipid	228	45.6%
PCB-180	0.2 µg/g lipid	182	36.4%
Sum PCB*	1.2 µg/g lipid	209	41.8%
Sum DDT + DDE	0.9 µg/g lipid	2	0.4%
Musk xylene	0.33 µg/g lipid	0	0%

*: 1.64 x (PCB138 + PCB 153 + PCB 180) to compare with German summation of a limited number of congeners

As for mercury, the range for each substance was very wide. The ratio between the highest and the lowest concentrations varied between 28 for HCB and 420 for PCB-118 (table 6.2.12).

The ratio of DDT to DDE was calculated in order to obtain some information about the origin of dioxins: If the share of DDT is higher, it would suggest a recent exposure to DDT through food contaminated by current use of DDT in nearby regions. If DDE dominates, this will indicate

that DDT has accumulated in the food chain over a longer period, possibly from far-away sources (AMAP, 1997). For the mothers of the study group only very low values for the DDT/DDE-ratio were found, thus indicating no direct contact to DDT. With the ban of DDT in the region in effect, this was to be expected. Still, the continued presence of DDT many years after the ban in the EU suggests that current or recent use of DDT elsewhere adds to the marine contamination.

6.2.3.4.3 Correlations between exposure data

The six different PCB congeners measured in breast milk were all significantly correlated to each other (Spearman's rho correlation coefficient >0.8 for most substances), therefore suggesting a common source for the contamination.

HCB, DDT, DDE, and trans-nonachlor also correlated very well with all PCB congeners: Σ PCB and HCB: $r=0.85$; Σ PCB and DDT: $r=0.85$; Σ PCB and DDE: $r=0.88$; Σ PCB and trans-nonachlor: $r=0.93$. However, methyl mercury and PCB congeners were related to each other to a much smaller degree (Σ PCB and MeHg: $r=0.39$). Also the correlation between the PCB-congeners and β -HCH was only weak (Σ PCB and β -HCH: $r=0.28$).

Musk xylene is correlated with DDE ($p=0.042$) and the sum of DDT + DDE ($p=0.048$). The higher the DDT/DDE-ratio the lower the concentration of musk xylene that was found in breast milk ($p=0.017$).

The DDT/DDE – ratio is higher in the milk of mothers with higher hair mercury, higher HCB, trans-nonachlor, PCB-105, PCB-118 ($p<0.01$ for all of them), and musk xylene ($p=0.013$).

Because the lipophilic contaminants originate from blubber and mercury from meat, the association demonstrates that mothers eating blubber are also likely to eat whale meat. However, in this regard the relatively short biological half-life of methyl mercury in the body (as compared to PCB and DDE) should be kept in mind.

The complete set of correlations can be found in table 6.2.22 in the appendix.

6.2.3.4.4 Parental characteristics and exposure values

Increased maternal age is significantly correlated with higher levels of β -HCH, HCB, all PCB-congeners, DDT, DDE, and trans-nonachlor (p -value <0.001). The association is likely due to accumulation of these persistent compounds with age. Higher parity also leads to an increase in mercury, HCB, all measured PCB-congeners, DDT, DDE, and trans-nonachlor (p -value for 2-tailed significance of Spearman's rho for the correlation: <0.001 for each of them). However, when controlling for maternal age, the picture changes: while mercury still increased significantly (p -value <0.001), β -HCH decreased with parity (p -value <0.001). For most other substances weak negative correlations were found.

A significant positive correlation was found for pre-pregnancy BMI with β -HCH, HCB (p -value 0.005 for Spearman's rho), musk xylene, PCB-105, sum of the three measured mono-ortho PCB-congeners (PCB-105, PCB-118, and PCB-156), DDT, and DDE, and for maternal weight with HCB, β -HCH, PCB-105, PCB-118, DDT, DDE, and musk xylene. But the data analysis did not reveal a significant correlation with maternal height.

A negative correlation appeared for weight gain during pregnancy with mercury, β -HCH, HCB, PCB-105, PCB-118, PCB-138, PCB-153, PCB-156, DDT, DDE, and trans-nonachlor.

Mercury in hair ($p=0.012$) and β -HCH ($p=0.003$) in breast milk were positively correlated with the mother's consumption of alcohol during pregnancy. The geometric mean of all other measured contaminants was higher in mothers who confirmed any consumption of alcohol during pregnancy. The ratio of DDT/DDE in these mothers was significantly higher than the one in teetotalers ($P=0.020$).

Smoking was associated with lower concentrations of musk xylene, PCB-105 ($p=0.070$), and DDT while the concentration of the other pollutants increased with the number of cigarettes smoked by the mother.

The mothers were interviewed about complaints about previous fertility problems. Those who reported problems with getting pregnant had significantly higher PCB-118 ($p=0.049$), PCB-138 ($p=0.016$), PCB-153 ($p=0.026$), PCB-156 ($p=0.030$), PCB-180 ($p=0.034$), DDE ($p=0.040$), and trans-nonachlor ($p=0.050$) than those who became pregnant without problems or delay. Mercury was lower ($p=0.003$) in mothers who had troubles with infertility.

The data showed a tendency to a higher rate of spontaneous early abortions with higher concentrations of mercury ($p=0.050$), HCB ($p=0.083$), PCB-118 ($p=0.021$), PCB-105 ($p=0.015$), DDE ($p=0.018$), and DDT ($p=0.024$). Cases of late abortion, i.e. during 16th to 28th week of gestation, more frequently occurred at higher mercury concentrations ($p=0.034$). The same tendency was seen for β -HCH and HCB ($p=0.092$ and 0.097 , respectively): higher contamination levels thus seem to increase the risk for abortions. On the other hand, age and parity may be important covariates that need to be considered.

Because previous infertility and the number of abortions is strongly correlated with maternal age, the connection between both variables and the exposure data was analyzed once more after controlling for maternal age. The significant correlations remained unchanged. Solely the significance of MeHg as protection against infertility vanished. Further exploration of these issues seems indicated.

For the relation between contaminants and complications during parturitions significant correlations were found for: HCB ($p=0.027$), β -HCH ($P=0.005$), PCB-105 ($p=0.033$), PCB-118 ($p=0.037$), PCB-138 ($p=0.035$), PCB-156 ($p=0.026$), and DDE ($p=0.036$). For the cases where complications during child birth were recorded, the concentrations of pollutants uniformly was higher than for those with an uncomplicated birth.

Significant correlations (p -values for 2-tailed significance of Spearman's rho are given in parentheses if they were ≤ 0.05 and ≥ 0.001 , all other p -values were < 0.001) were found between mother's education, profession, and present occupation with the exposure parameters. Higher maternal education lead to lower mercury (0.047), higher β -HCH (0.001), PCB-105 (0.006), PCB-118 (0.007), PCB-138 (0.011), PCB-153 (0.005), PCB-156 (0.001), PCB-180 (0.001), DDT, DDE (0.007), trans-nonachlor (0.027), and musk xylene (0.034) is found with higher maternal profession; higher maternal occupation

is significantly correlated with higher HCB (0.005), β -HCH, PCB-105 (0.012), PCB-118 (0.018), PCB-138 (0.009), PCB-153 (0.003), PCB-156, PCB-180, DDT (0.008), DDE (0.039), and trans-nonachlor (0.022).

If the same analyses were done for the father / the partner of the mother, the results were: higher school education of father is correlated with a lower MeHg in mother's hair (0.016), with a lower HCB (0.020), PCB-105 (0.002), PCB-118 (0.003), PCB-138 (0.002), PCB-153 (0.005), PCB-156 (0.045), PCB-180 (0.026), DDE (0.008), and trans-nonachlor in mother's milk; higher values of HCB (0.012), β -HCH (0.020), PCB-105 (0.023), PCB-118 (0.008), PCB-138 (0.015), PCB-153 (0.006), PCB-156 (0.010), PCB-180 (0.004), DDT, DDE (0.003), and trans-nonachlor (0.025) correlate significantly with a higher score for father's profession, while for father's occupation significant correlation could be found solely for β -HCH (0.011), PCB-118 (0.047), and DDT (0.003).

Overall, these associations perhaps reflect a lower educational level in subjects involved in whaling, perhaps also in the degree that governmental advisories are taken into regard. Further studies are needed to clarify these aspects and their associations with age.

When exposure data were inspected with respect to mother's nationality, contaminants were higher in the samples of Faroese women. Only β -HCH tended to be slightly lower in Faroese women ($p=0.129$). The differences in exposure were highly significant for all measured PCB-congeners, trans-nonachlor, and DDE (2-tailed significance of results of T-test for independent samples test: <0.001). These differences were not explained by confounding by maternal age, weight, BMI and / or parity. The impact of the father's nationality was much weaker and not significant.

TABLE 6.2.14 SUMMARY OF CORRELATIONS BETWEEN CONTAMINANTS AND PARENTAL CHARACTERISTICS

	Positive	Negative
Mercury in (mother's hair)	Parity**, mother's alcohol consumption during pregnancy*, mother is Faroese**	Teetotal mother*, father's school education, weight gain during pregnancy*
β-HCH (in breast milk)	Maternal age**, mother's weight**, BMI**, mother's alcohol consumption during pregnancy**, father's profession*, father's occupation*, previous infertility*, complication under birth**	Weight gain during pregnancy, teetotalling mother**, mother is Faroese**, weight gain during pregnancy*, OOS**
HCB (in breast milk)	Maternal age**, mother's weight**, BMI**, father's profession*, mother is Faroese*, parity**, complication under birth*	Father's school education, weight gain during pregnancy**, OOS*
Σ PCB (in breast milk)	Maternal age**, parity**, father's profession**, mother is Faroese**, previous infertility**, complication under birth*	Father's school education**, weight gain during pregnancy**, OOS*
4,4'-DDT (in breast milk)	Maternal age**, mother's weight**, BMI**, father's profession**, father's occupation**, mother is Faroese**, parity**, complication under birth*	Weight gain during pregnancy**
4,4'-DDE (in breast milk)	maternal age**, mother's weight*, BMI**, parity**, father's profession**, mother is Faroese**, previous infertility*, complication under birth*	Father's school education**, weight gain during pregnancy*, OOS*
Trans-nonachlor (in breast milk)	Maternal age**, parity**, father's profession*, mother is Faroese**, previous infertility**	Father's school education**, weight gain during pregnancy*, OOS**
Musk xylene (in breast milk)	Mother's weight**, BMI**	

** : Correlation is significant at the 0.01 level (two-tailed).

* : Correlation is significant at the 0.05 level (two-tailed).

6.2.3.5 Correlations between exposure variables and diet

In this section, the association between dietary habits is examined with regard to contamination with particular pollutants.

The highest correlation between mercury in mother's hair and food was found for the number of meals containing whale meat. But also blubber consumption, the number of fish meals per week, and the number of fulmars eaten during pregnancy showed a very close correlation to hair mercury of the mother (table 6.2.23). However, these dietary habits were also intercorrelated.

HCB, DDT, DDE, and trans-nonachlor were very closely correlated with the consumption of blubber and fulmar. The concentration of these pollutants also increased significantly with the consumption of whale meat and the meat of other seabirds. The amount of DDT ingested with blubber seems to be higher than that of DDE resulting in a significant increase of the DDT/DDE-ratio in the milk of these mothers (table 6.2.23). A detailed assessment of the

contribution of these dietary items will depend on data on pollutant concentrations in these items.

Concentrations of PCB-105, 118 and 138 in breast milk are very well correlated to the consumption of any form of whale products (meat or blubber, dried or fresh) and the number of fulmars eaten during pregnancy. The consumption of blubber (dried or fresh) and fulmars seem to best predict the concentration of the PCB-congeners 153, 156, and 180 in breast milk, while whale meat is only a minor source of these congeners (table 6.2.24).

No significant positive correlation was observed between any kind of food mentioned on the questionnaire and β -HCH or musk xylene.

The consumption of which food leads to a significant change of the load with the different pollutants? Information to the question is collected in table 6.2.15. Dried blubber and fulmar seem to be the two sources that add the highest number of contaminants in significant amount. Not all possible foods containing the measured pollutants were registered in the questionnaire. More information about the sources of the contaminants may be revealed by further research.

TABLE 6.2.15 CORRELATIONS BETWEEN DIETARY HABITS AND EXPOSURE VARIABLES

Dietary habit	Positive correlation with harmful substances (unless otherwise indicated)
Number of whale meals	Hair mercury**, HCB, PCB 118, PCB 105**, PCB 138, DDE, DDT**, t-nonachlor**
Total amount of whale meat	Hair mercury**, PCB 118, PCB 105, DDE, DDT**, t-nonachlor**
Number of blubber meals	PCB 118, PCB 105, PCB138, Σ PCB**, DDE, DDT*, ratio DDT/DDE, t-nonachlor**
Estimated total amount of blubber	Hair mercury**, PCB 118, PCB 105, PCB 138, Σ PCB**, DDE**, DDT**, ratio DDT/DDE, t-nonachlor**
Number of other meals with dried blubber	Hair mercury**, PCB 118**, PCB 153, PCB 105, PCB 138**, sum of PCBs, DDE**, DDT**, ratio DDE/DDT, t-nonachlor**
Number of fish meals per week	Hair mercury**, PCB 105, PCB 118, DDT, DDE, t-nonachlor
Meals with fulmar	HCB, PCB 118**, PCB 153**, PCB 105**, PCB 138, PCB 156**, PCB 180, Σ PCB, DDE, t-nonachlor**
Meals with other seabirds	HCB, PCB 118, PCB 105, DDT
Number of other meals with meat per week	Negative correlation to: hair mercury**, HCB, HCH

** Correlation is significant at the 0.01 level (2-tailed); all other correlations were significant at the 0.05 level.

6.2.3.6 Dependent variables, part I: Anthropometric data, gestational age, and gender

6.2.3.6.1 Anthropometric data of infants

6.2.3.6.1.1 Description

A wide range of birth weight was found with a Gaussian distribution. Boys were heavier than girls at birth, they were longer, and had a bigger head circumference (length and head circumference were measured at the neurological examination). The differences of all three measurements between both sexes were highly significant (p-value <0.001) (table 6.2.16).

TABLE 6.2.16 ANTHROPOMETRIC DATA (MEAN, SD, SECOND LINE IN EACH CELL: RANGE OF PARAMETER)

	All infants	Boys (N=267)	Girls (N=233)
Birth weight in g (N=500)	3743 ± 473 2450 - 5400	3827 ± 505 2450 - 5400	3647 ± 415 2650 - 5100
Length at NOS in cm (N=499)	54.3 ± 1.9 47 - 60	54.8 ± 1.9 47 - 60	53.7 ± 1.7 50 - 59
Head circumference at NOS in mm (N=498)	370 ± 12 340 - 401	373 ± 11 342 - 401	366 ± 11 340 - 395

6.2.3.6.1.2 Correlations

6.2.3.6.1.2.1 Correlations of anthropometric variables and gestational age with parental attributes

The birth weight of infant was positively correlated with BMI of the mother ($p=0.002$), weight gain during pregnancy ($p=0.048$), mother's weight ($p<0.001$), mother's height ($p<0.001$), mother's age ($p=0.020$), parity ($p<0.001$), and the fact that the mother suffered from diabetes (persistent or gestational) ($p=0.013$). A reduction of birth weight was linked to maternal smoking ($p=0.003$) and arterial hypertension ($p=0.041$). The analyses about a correlation between the metric data (mother's weight, height, BMI, age, parity, and weight gain during pregnancy) were repeated while controlling for gestational age in days. The results remained virtually unchanged.

Birth weight is higher in infants of Faroese mothers as compared to those of non-Faroese mothers ($p=0.002$). The head circumference of these infants was also bigger ($p=0.033$) while the difference in length did not reach significance.

No major difference in these correlations was seen between boys and girls.

Gestational age (in days) increased with a higher maternal weight ($p=0.030$). Alcohol consumption during pregnancy appeared to prolong gestation ($p=0.011$). Arterial hypertension already present before pregnancy ($p=0.043$) and hypertension early in pregnancy ($p=0.016$) led to a shorter gestation. These tendencies are obviously affected by obstetric interventions.

6.2.3.6.1.2.2 Correlation of anthropometric outcome and gestational age to exposure variables

A positive correlation between all exposure variables and birth weight were observed except for β -HCH. A significant positive influence on birth weight was reached for PCB-105 and PCB-118, and the sum of the three measured mono-ortho PCBs (congeners 105, 118, and 156). To differentiate between possible effects of pollutants on birth weight, the data were re-analyzed while controlling for gender. As already mentioned, the birth weight of boys was significantly higher than that of girls (see table 6.2.16). The boys showed a high sensitivity to HCB, PCB-105, PCB-118, and DDT, while for girls no significant effect of exposure variables on birth weight could be found (table 6.2.25 in the appendix). For boys a highly significant relation between the DDT / DDE-ratio and birth weight was seen: the higher the ratio, the higher the birth weight ($p=0.003$).

No new information was achieved if partial correlation coefficients were calculated while controlling for gestational age, maternal age, parity, and log10 of mother's BMI.

Effects of exposure on head circumference measured at the neurological examination were weak. (table 6.2.26 in the appendix). If the analysis for the correlation between head circumference at neurological examination and exposure data was repeated after controlling for corrected age at neurological examination, a significant positive influence of MeHg, PCB- 105, and PCB- 118 was seen for the whole cohort, while only PCB-105 increased the head circumference in boys (table 6.2.27). When taking the percentile of head circumference, the impact of all measured contaminants is greater in boys. HCB, PCB-118, 105 and 138, sum of the three mono-ortho PCBs, DDT, and trans-nonachlor reached significant levels of correlation. In girls no significant correlations between either head circumference – controlled for age – or percentile of head circumference at NOS and exposure data were found (table 6.2.28 in the appendix).

For the complete cohort, no significant correlation was found between length at NOS and exposure data. All correlations were, however, positive except for β -HCH which gave a weak negative correlation (table 6.2.29 in the appendix). After further controlling body length for corrected age after expected date of delivery at neurological examination, for maternal height and weight, parity, and for log 10 of PCB-105, the relation between length and exposure data became more clear: HCB, β -HCH, DDT, and DDE significantly decreased the length (table 6.2.30).

6.2.3.6.1.2.3 Linear regression model for calculation of birth weight

All the identified major factors influencing birth weight were used to create a linear regression model. The log10-transformed values of PCB-105 and Σ PCB served as independent variables. Gestational age (in days), maternal height, weight, and parity were introduced as covariates. As further important factors for birth weight, sex, Faroese and non-Faroese mother, mother with and without diabetes mellitus, and smoking of mother during pregnancy were added. For a summary of the model see table 17. The R^2 for the model was 0.274 (adjusted R^2 0.259).

TABLE 6.2.17 LINEAR REGRESSION FOR BIRTH WEIGHT (IN GRAMS)

	Unstandardized coefficient B	Standard error of B	Significance for T	95%-confidence interval for B
(Constant)	-2984	839	<0.001	-4633 – -1335
Gestational age (days)	18	2	<0.001	13 – 22
Height of mother (cm)	9	3	0.006	3 – 16
Weight of mother (kg)	5	2	0.012	1 – 9
Primipara (no / yes)	-256	42	<0.001	-339 – -173
Log10 PCB 105	242	78	0.002	87 – 396
Log10 PCB 153	-315	108	0.004	-527 – -103
Gender (male / female)	-188	37	<0.001	-260 – -115
Smoking/non-smoking mother	-110	41	0.008	-191 – -28
Non-Faroese / Faroese mother	351	103	0.001	148 – 554
Diabetes of mother no / yes	155	65	0.018	27 – 283

Unstandardized regression coefficients can be used to calculate the change of birth weight (in grams) per unit change in each variable, e.g. if gestation is one day longer, the birth weight can be expected to be 18g higher. For the

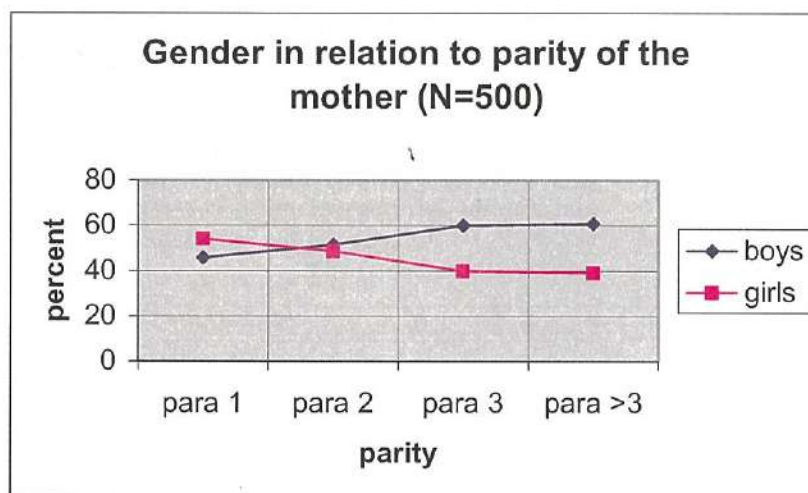
log10-transformed exposure variables, a 10-fold change will cause a change of birth weight in the magnitude of the correlation coefficient, i.e. a plus of 221g for a 10-fold increase in PCB-105 and a minus of 315g for Σ PCB. The results shown indicate that several factors impact on birth weight. The effect of PCB congeners in different directions may be a statistical phenomenon due to collinearity, but it is also possible that PCB congeners may exert different effects.

6.2.3.6.2 Gestational age

Duration of gestation was mostly influenced by maternal condition during the present, but also during former pregnancies: Arterial hypertension in the first trimester of pregnancy as well as any complication during a previous pregnancy led to a significantly earlier delivery (4 resp. 2 days). Other non-obstetrical disease during the present pregnancy (excluding diabetes, epilepsy, hypertension, and heart diseases) was also correlated with a significant shortening of gestation (mean of gestation for healthy mothers: 284 days, for those with disorders: 280 days; $p=0.039$). Previous spontaneous abortion (one or more) resulted in a shorter gestation for the child: 284 days if no abortion was reported, 282 days for any number of early abortions <16 weeks ($p=0.025$). The same difference in duration of gestation was found for primiparae versus women with at least one previous birth ($p=0.031$). For the next pregnancies (third or more deliveries) a significant additional shortening of gestation was found ($p=0.024$). Exposure variables were only weakly associated with gestational age.

FIGURE 6.2.2: DISTRIBUTION OF SEX FOR PARITY

$R^2=0.9$



6.2.3.6.3 Sex

Mothers of boys were about one year older than those of girls ($p=0.046$). This might explain why all measured contaminants were found in a slightly higher concentration in boys except for β -HCH that was higher in girls. None of the differences reached significance level. Since parity is also known to be of major influence on the load of contaminants, the infants were grouped after parity of mother. The sex distribution shows a higher ratio of girls for primipara (opposed to general experience which predicts a higher ratio of boys) and it changes after the second pregnancy to the expected distribution. To confirm this strange finding, the sex of all infants with known parity and who were born in the Tórshavn hospital during a period of 28 months

(15.11.1997 to 15.3.200, N=1251) were analyzed, but the result remained unchanged (R^2 of trend line was 0.89). (Fig. 6.2.2). It is still unclear whether this tendency is accidental or related to some form of selection bias.

6.2.3.7 *Dependent variables: neurological outcome*

Analysis of neurological outcome in relation to exposure variables is divided in the results for the neurological optimality score (NOS) and the analysis of further neurological signs which were measured as a part of the neurological examination such as abnormal high or low muscle tonus, reduced amount of spontaneous movements (hypokinesia) and so on.

6.2.3.7.1 Neurological outcome, part I: NOS

In general the total score of the NOS became higher towards the end of the examination period (correlation between date of examination and NOS highly significant, p-value for the Pearson correlation was <0.001). For the first 250 infants the mean of the NOS was 49.6 points out of a maximum of 60 points, for the second half of the children 50.3.

6.2.3.7.1.1 *Description of results*

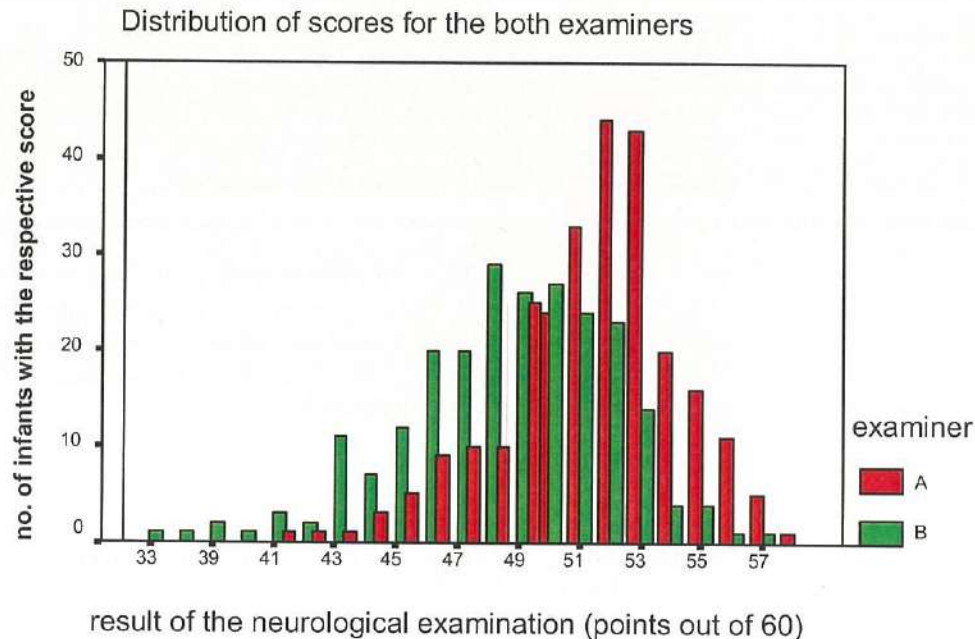
Most of the children, i.e. 368 or 73.6% were examined at the optimal age for the neurological examination (between 10 and 21 days after the expected date of birth). A total of 33 children (6.6%) were younger than 10 days, and 99 (19.8%) older than 3 weeks (see figure 6.2.4 in the appendix). This variation in age is obviously an effect of scheduling problems for mothers not residing in Tórshavn.

The examination started in four children directly after the last meal. The largest interval between last meal and examination was 5 hours. The majority of children (323 / 467 or 69.2%, for 33 infants this value was unknown) was seen between 1½ and 3 hours after the last meal.

262 children were examined by pediatrician A, 233 by pediatrician B. The remaining 5 infants were seen by pediatrician C. The mean as well as the median of the sum score was 50 (out of 60) with a standard deviation of 3.6. The child with the lowest score archived 33 points, the highest score was 58. P10 was 45, P25: 48, P75: 52, P90: 54. For 8 infants (1.6%) the clinical diagnostic classification was suspect, none of the infants were considered abnormal. The clinical diagnosis sorted after examiner were: 262 / 262 children seen by A were normal, 226 / 233 children seen by B were normal, 7 abnormal. C examined 5 children, one of them was classified as suspect.

As already mentioned in paragraph 6.2.3.2.1, the result of the NOS was significantly higher in infants, examined by A. The distribution of the scores given by the two main examiner can be seen in figure 6.2.3.

FIGURE 6.2.3: DISTRIBUTION OF NEUROLOGICAL OPTIMALITY IN DEPENDENCE OF EXAMINER (A/ B)



6.2.3.7.1.2 Association / correlation with parental / gestational data

A negative correlation of NOS was found with the percentile of head circumference ($p=0.012$) and percentile of length ($p=0.010$), i.e. infants with a larger head or longer length scored lower. In the subcohort of infants seen by B the large-for-gestational-age infants (percentile of birth weight >90) had a significantly lower NOS than infants with a birth weight that was small or appropriate for gestational age ($p=0.021$).

Parental smoking (sum of score for maternal and partner's smoking during pregnancy) is correlated to a less favorable diagnosis of NOS (suspect or abnormal result of neurological examination) ($p=0.036$).

The higher the score for mother's education (or the higher one of the scores for parents school education) the more likely was the diagnoses "normal" in the summary for the neurological examination ($p=0.034$; for parents' education, 0.009). If the result of NOS in infants whose parents had a higher level of profession or occupation (see paragraph 6.2.2.5.5) were compared to those with a lower level of profession or education, there was a significant decrease in NOS ($p=0.017$).

In the B-subcohort, infants who were large-for-gestational age (birth weight $>P90$) had a significant lower result in the neurological examination (mean of NOS for large-for-gestational age infants: 47.2, appropriate- or small-for-gestational age: 48.7; $p=0.021$). This findings will be further analysed, and regard will be taken of the potential risk of birth complications associated with large birth size.

6.2.3.7.1.3 Correlation of NOS with exposure data

An increase in concentration of 4,4'-DDT in breast milk led to a less favorable NOS diagnosis, i.e. more frequently a suspect result was given ($p=0.026$). No other significant correlations between exposure and NOS could be found in the complete group.

In the children examined by B, clear correlations of the NOS with the exposure data were seen: PCB-118, PCB-105, DDT, and trans-nonachlor led with high significance to a lower NOS. Also HCB, PCB-138, PCB-180, and DDE caused a reduction of NOS, the connection between NOS and contaminants being significant ($p<0.05$). For full detail of correlation coefficients and level of significance: see table 6.2.18.

TABLE 6.2.18 CORRELATION OF NOS WITH EXPOSURE DATA, DIFFERENTIATED FOR EXAMINER

	All children (N=500)		Subcohort B (N=233)		Subcohort A (N=262)	
	Spearman's rho	P-value	Spearman's rho	P-value	Spearman's rho	P-value
Methylmercury	-0.061	0.174	-0.094	0.151	-0.013	0.838
HCB	-0.064	0.151	-0.206**	0.002	0.077	0.214
β -HCH	-0.020	0.651	-0.101	0.125	0.038	0.543
Musk xylene	-0.019	0.664	-0.077	0.244	0.028	0.657
PCB 118	-0.033	0.457	-0.176**	0.007	0.104	0.094
PCB 153	-0.036	0.428	-0.150*	0.022	0.093	0.135
PCB 105	-0.079	0.077	-0.220**	0.001	0.064	0.303
PCB 138	-0.018	0.682	-0.143*	0.030	0.114	0.066
PCB 156	-0.006	0.894	-0.107	0.103	0.118	0.057
PCB 180	-0.030	0.497	-0.150*	0.022	0.109	0.078
Σ PCB	-0.028	0.535	-0.151*	0.022	0.107	0.083
Sum of MO-PCB ^{&}	-0.035	0.432	-0.173**	0.008	0.106	0.088
4,4'-DDE	-0.027	0.545	-0.158*	0.016	0.099	0.111
4,4'-DDT	-0.082	0.066	-0.210**	0.001	0.061	0.323
Trans-nonachlor	-0.023	0.603	-0.170**	0.010	0.124*	0.046

* : Significant correlation (2-tailed significance)

** : Highly significant correlation (2-tailed significance)

& : Sum of three mono-ortho PCBs (105+118+156)

The correlation between NOS and exposure data were also calculated while controlled for corrected age at neurological examination, for environmental temperature, for interval since last meal, and for weight gain of mother during pregnancy. Boys reacted more strongly in respect to their neurological performance to the PCB-congeners, to DDT, DDE, and trans-nonachlor. For HCB, a highly significant negative correlation with the NOS was found in boys while the substance had no apparent influence in girls. The significant negative effect of β -HCH in boys converted in girls into a significant positive factor for the NOS. Mercury acted as a weak positive factor on NOS in boys while it decreased the performance in girls, nearly reaching significant level of correlation. The complete list of correlations and p-values can be found in table 6.2.31 in the appendix.

6.2.3.7.1.4 *Linear regression model for calculation of NOS*

The list of influences on the neurological status of a newborn infant is long. Therefore models that show the effects of prenatal contaminants on the neurological presentation have to be very complex. They will include many factors. The summary of one model using multiple linear regression is presented in table 6.2.19. As in table 6.2.17, the change of one unit of a variable will cause a change of the NOS in the magnitude of the respective Beta-coefficient (for log10-transformed variables, a change with the factor 10 of the variable will cause a change of one unit of the NOS). R^2 was 0.221, adjusted R^2 0.198. For the children examined by B another model was constructed which gave a R^2 of 0.273 (adjusted R^2 : 0.228), see table 9.2.7 in the appendix. If in this subcohort only the boys were tested, the R^2 came up to 0.566 (adjusted R^2 0.514) while the same model did not fit for girls again suggesting that different mechanism are responsible for the performance in boys and girls.

TABLE 6.2.19: LINEAR REGRESSION MODEL FOR NOS (FOR CHILDREN SEEN BY A AND B)

MODEL SUMMARY

	R	R ²	Adjusted R ²	Standardized error of the estimate
All children	0.556	0.309	0.284	2.99
All boys	0.595	0.354	0.311	2.99

	Unstandardized coefficients		Standardized coefficients	Significance		Collinearity statistic (tolerance)
	Beta	Standard error	Beta	♀+♂ (N=495)	♂ (N=266)	
Constant	44.73	3.960		<0.001	<0.001	
Examiner (A /B)	-2.776	0.336	-0.392	<0.001	<0.001	0.732
Date of examination	0.001	0.001	0.092	0.030	---	0.927
Interval of examination to last meal (min)	0.006	0.003	0.087	0.063	0.136	0.755
Stability of states (no / yes)	3.063	0.500	0.256	<0.001	<0.001	0.944
Time between rupture of membranes and birth (min)	-0.027	0.019	-0.060	0.153	0.241	0.955
Apgar score at 5 minutes	0.691	0.327	0.089	0.035	0.033	0.938
Neonatal illness (no / yes)	0.486	0.364	0.056	0.182	0.113	0.940
Level of higher one of parents' profession or occupation (low / high)	-0.902	0.308	-0.128	0.004	0.003	0.873
Mother's age (years)	0.067	0.032	0.098	0.035	0.010	0.770
Maternal weight (kg)	-0.031	0.015	-0.091	0.032	---	0.913
Weight gain during pregnancy (kg)	-0.044	0.031	-0.062	0.148	---	0.918
Early abortions (no / yes)	-0.676	0.342	-0.082	0.049	0.010	0.959
Other non-obstetrical disease of mother (no/yes)	1.178	0.651	0.076	0.071	0.177	0.931
Log10 of PCB 156	4.370	1.758	0.386	0.013	0.048	0.069
Log10 of PCB 180	-5.168	1.757	-0.460	0.003	0.028	0.067
Log10 of β-HCH ^{&}	---	---	---	---	0.008	---
Log10 of musk xylene ^{&}	---	---	---	---	0.032	---

* : Not in model for boys

& : Not in model for all children

It is noteworthy that two PCB congeners caused effects in different directions. Collinearity problems limit the validity of this findings. However, in conjunction with the associations with birthweight, the possibility exists that dioxin-related PCB congeners have toxic potentials that differs from other PCBs.

6.2.3.7.1.5 Correlation between diet during pregnancy and neurological status at 2 weeks

For the combined cohort no relation between the result of the neurological examination and one of the recorded dietary habits could be shown. However, the reflex cluster score improved with a higher number of meals with dried blubber and the total amount of blubber, consumed during pregnancy, but the consumption of fulmar led to a reduction of the score. Seabirds but not fulmar caused a significant reduction of the postural tone cluster score (table 6.2.10).

When the analyses were repeated for the subcohort of children examined by A and the one seen by B, the results did not differ much. Additionally a highly significant correlation was found between the number of fish meals per week and the NOS ($p=0.008$) for subcohort B. Diverging from the result of the combined cohort, the girls examined by B showed a very strong reduction of the reflex cluster score with an increasing number of meals with or total amount of blubber ($p=0.007$ and 0.008).

TABLE 6.2.20 SPEARMAN'S RHO AND P-VALUES (IF P-VALUE WAS ≤ 0.10)

	NOS	Reflex cluster score	Postural tone cluster score
Number of whale meals during pregnancy (N=261)	N.s.	N.s.	N.s.
Total amount of whale meat (in g) consumed during pregnancy (N=217)	N.s.	N.s.	N.s.
Number of blubber meals (N=256)	N.s.	0.117 0.063	N.s.
Total amount of blubber (in g) (N=238)	N.s.	0.140 0.030	N.s.
Number of other meals with dried whale meat (N=257)	N.s.	0.110 0.079	N.s.
Number of other meals with dried blubber (N=258)	N.s.	0.212 0.001	N.s.
Number of fish meals per week (N=256)	N.s.	N.s.	N.s.
Number of meals with fulmar (N=258)	N.s.	-0.139 0.026	N.s.
Number of meals with other seabirds (N=257)	N.s.	N.s.	-0.153 0.014
Total number of seabirds (N=256)	N.s.	-0.136 0.029	-0.172 0.006
Number of other meals with meat (per week) (N=253)	N.s.	N.s.	N.s.

6.2.3.7.2 Neurological outcome, part II: other neurological signs

6.2.3.7.2.1 Hypokinesia

Interval between last meal and neurological examination and several exposure variables weakly increased the probability to get the diagnose

“hypokinesia” in the summary of the neurological examination. A higher concentration of mercury leads apparently to more activity: the diagnose “hypokinesia” was given less frequently ($p=0.119$; for boys only 0.038). PCBs, 4,4'-DDE, and trans-nonachlor showed stronger effects in girls. For details: see table 6.2.33 in the appendix.

6.2.3.7.2.2 *Postural tone cluster score*

Significantly lower results of postural tone cluster score were achieved by children with icterus at neurological examination (mean for the children without icterus: 8.2 versus 6.5 for those with icterus; $p=0.001$). In children of Faroese mothers, the tone cluster was significantly lower than in those of foreign mothers (mean 8.1 versus 9.1; $p=0.034$). No influence of the father's nationality was found. A significantly higher postural tone cluster score ($p=0.020$) was found in infants who needed transfer to the neonatal ward for observation or treatment directly after birth (muscle hypertonia caused by mild asphyxia?).

When testing all boys, a negative influence on the result of the postural tone cluster score of all measured PCB-congeners, DDT, DDE, HCB, β -HCH, and trans-nonachlor was seen. For girls the tonus cluster score increased with increasing β -HCH (Spearman's ρ 0.132, p -value 0.045), the same was true for the PCB-congeners, HCB, dioxins, trans-nonachlor. For mercury a mild negative correlation with the tonus score of girls was found ($p=0.212$). Testing the partial correlations for postural tone cluster score (controlled for corrected age at neurological examination, environmental temperature, interval to last meal, and weight gain), for the combined cohort a very weak negative influence of all substances was found. The separate analysis of boys and girls revealed obvious differences in their reaction to the exposure variables: while mercury was negatively correlated to the tone score in girls, all other substances slightly improved the result. In boys, for all contaminants a negative correlation to the tone cluster was seen, the strongest the one with PCB-118 ($p=0.105$). The negative influence of mercury on the tonus in boys was much weaker than in girls.

If only the tone of the legs was tested in the B-subcohort a significant negative influence of MeHg could be shown for the whole group ($p=0.021$) as well as for the girls ($p=0.032$). No clear influence of exposure data on the muscle tonus of the legs in the children examined by A.

6.2.3.7.2.3 *Opisthotonus*

Pediatrician A never found opisthotonus in the infants examined, so only the subcohort B was used. Children with the diagnosis “opisthotonus” had significantly lower levels of PCB-118 ($p=0.030$), PCB-153 ($p=0.012$), PCB-138 ($p=0.015$), PCB-156 ($p=0.024$), PCB-180 ($p=0.012$), DDE ($p=0.019$), and trans-nonachlor ($p=0.028$) in breast milk. In girls the apparent preventive effect of the named pollutants against the diagnose “opisthotonus” was stronger than in boys.

6.2.3.7.2.4 *Reflex cluster score*

When the complete cohort was analyzed, all of the POPs except musk xylene showed a weak negative influence on the reflex cluster score. When the correlation was controlled for corrected age at NOS, environmental temperature, interval to last meal, and maternal weight gain during pregnancy the negative correlation between β -HCH and HCB with the reflex cluster

score became significant ($p=0.029$ and 0.031). As for the postural tone cluster score the separate analysis of boys and girls revealed differences in their reaction to the exposure variables: Mercury was positively related with the reflex cluster score in boys, but negatively in girls. The observed negative correlations between all of the POPs in boys were much stronger (especially for DDT) than in girls.

The bivariate correlation of the reflex score with the contaminants were also tested for the two subcohorts. For the subcohort A a positive correlation between reflex score and all exposure data, including mercury was found, but none of the correlations reached significance (all p -values >0.10). An uniform negative relation between all exposure data and the reflex cluster score were found in the children examined by B. Beside mercury and musk xylene the correlations were significant for β -HCH, PCB-118, PCB-153, PCB-138, PCB-180, and trans-nonachlor (see table 6.2.34 in appendix).

6.2.3.8 *Different reactions in boys and girls*

Here a short summary of the diverging reactions on the different pollutants in boys and girls will be given. First the effects on growth are discussed. Then the results of the neurological examination in dependence of gender follow.

PCB-105 had the strongest impact on birth weight, length and head circumference. In boys, the positive correlation between the concentration of this congener in breast milk was much higher than in girls. When the exposure of the other pollutants was controlled for PCB 105, all visible influences were still greater in boys: percentile of head circumference increased more with higher concentrations of contaminants than in girls; length was reduced more (except for DDT which affected girls more than boys) by all pollutants in boys.

Mercury had a "positive" influence on the NOS in boys, an adverse one in girls. β -HCH led to a lower NOS in boys, to higher one in girls. In both cases the correlation was a significant one. HCB didn't influence the neurological outcome in girls, while in boys a significant negative influence was observed. The negative impact of all tested PCB-congeners (IUPAC numbers 105, 118, 138, 153, 156, and 180) was much weaker in girls than in boys. The same was true for DDE, DDT, and trans-nonachlor.

The diagnosis "opisthotonus" was given less frequently with higher exposure to contaminants. This effect was stronger in girls than in boys, but musk xylene in girls leads to opisthotonus in girls, while seemingly preventing it in boys.

Reflex cluster score improved with increasing maternal hair-mercury in boys, while becoming lower in girls. All other contaminants showed a stronger negative impact on reflex cluster score in boys, especially DDT and β -HCH.

The correlation of hypokinesia with pollutants was more marked in girls for all measured PCB congeners, for DDE, DDT, and trans-nonachlor. On the other hand, boys tended more frequently to obtain the diagnosis "hypokinesia" with higher β -HCH and musk xylene.

A list of the observed differences in the neurological outcome in boys and girls is given in table 6.2.35.

6.2.4 Discussion

In considering the findings of the present study, the complexity of the exposure situation must be appreciated. More than 200 PCB-congeners exist. The number and position of the chlorine is responsible for the toxicity of a particular congener. Usually sums of PCB-congeners were used. They were introduced to reflect the total toxicity – including dioxin-like effects – of a certain mixture by adding few of the measurable congeners. But the mixtures found in seafood will vary depending on the sources. Therefore, the special effects of particular congeners such as PCB-105 reported here, might easily have been overlooked. The same difficulty arises for some of the other POPs where only few substitute substances are measured. Other, until now unknown substances might accompany the POPs in whale products, fish, or other specific food (seabirds) eaten by Faroese mothers. Thus the measured substances could function as a surrogate variable for the unknown substance. If the concentrations of the measured and the surrogate substance are not strongly correlated, interpretation of results becomes difficult. Some other habits, dietary or otherwise, which were not recorded in the present study, might also be the origin of an exposure which causes results that contradict the conclusions of other studies. Only further research, especially the examination of the effect of the particular PCB-congeners, will help to resolve these problems.

6.2.4.1 *Characteristics of parents, life style factors, dietary habits of mothers and exposure data*

Several correlations between maternal attributes and the load of POPs have been described in the literature:

The positive correlation of the concentration of all substances with maternal age was already reported in 1984 (Fein et al., 1984). In Germany, values for contaminants in a 40-years old primipara are about 1.5 to 2.0 higher than in a 20-year old mother of a first child (Institut für Wasser-, Boden- und Lufthygiene des Bundesunweltamtes, 1999). There is a simple explanation for this phenomenon: the amount of organochlorines in body fat increases with age because of continuous human exposure to low levels and long biological half-lives of these chemicals (Schade et al., 1998; Vartiainen et al., 1998). In our cohort, maternal age was highly significantly correlated to all of the organochlorines.

Higher age and higher parity are connected with each other. While some authors found a negative correlation of exposure data to parity (Fein et al., 1984), in another Faroese cohort increased parity or age were associated with slightly increased mercury concentrations in cord blood and hair (Weihe et al., 1996). In a Finnish study, PCB concentrations in human milk in primiparae increased with age (Vartiainen et al., 1998). Breast-feeding of previous children can result in a decrease of body fat depots and it constitutes an important route of excretion for persistent organochlorines; the same is true for previous pregnancies (Fein et al., 1984; Schade et al., 1998). Not knowing how long the mothers nursed their children and lacking any information about weight gain or loss of weight during these period(s), exposure data were analyzed for the present cohort with respect to maternal age and parity. Not all of the organochlorines were reduced by a higher number of pregnancies and thus – for most of the Faroese mothers will nurse their infants for at least half a year – longer total period of breast-feeding.